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(21) International Application Number: PCT/GB91/00163 (22) International Filing Date: 4 February 1991 (04.02.91) (30) Priority data: 9002387.0 2 February 1990 (02.02.90) GB (71) Applicant (for all designated States except US): IMPERIAL COLLEGE OF SCIENCE, TECHNOLOGY & MEDICINE[GB/GB]; Sherfield Building, Exhibition Road, London SW7 2AZ (GB). (72) Inventors; and (75) Inventors/Applicants (for US only) : THOMAS, Howard, Christopher [GB/GB]; 39 Beech Drive, London N2 9NX (GB). KARAYIANNIS, Peter [GB/GB]; 54 Townsend Avenue, London N14 7HJ (GB).		(74) Agent: GADSDEN, Robert, Edward; 3i Research Exploitation Limited, The Gate House, 2 Park Street, Windsor, Berkshire SL4 1LU (GB). (81) Designated States: AT (European patent), BE (European patent), CA, CH (European patent), DE (European patent), DK (European patent), ES (European patent), FR (European patent), GB (European patent), GR (European patent), IT (European patent), JP, LU (European patent), NL (European patent), SE (European patent), US. Published <i>With international search report. Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i>
(54) Title: HEPATITIS A VACCINES (57) Abstract An isolated polypeptide comprising a substantially antigenic part of at least one hepatitis A virus epitope, the polypeptide being free-from infectious material. The polypeptide is the expression product of a DNA molecule which has been incorporated into a virus, preferably vaccinia virus, using recombinant DNA technology. This expression product, of the recombinant virus containing the DNA molecule coding for it, may be incorporated into a vaccine for immunising against hepatitis A virus.		

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Hepatitis A vaccines.

This invention relates to the prevention of hepatitis and is particularly concerned with the production of new materials suitable for incorporation into a vaccine for the prevention of hepatitis A.

5 Infection with hepatitis A virus (HAV) remains an important cause of morbidity and occasionally mortality, both in countries where it is endemic and in the industrialised nations where it is normally sporadic. As sanitary conditions have been improving in developing
10 countries, the prevalence of HAV seropositivity has been decreasing so creating a large pool of susceptible individuals. These individuals together with high risk groups such as day-care centre staff, parents and siblings of toddlers attending such centres, promiscuous
15 homosexual men and military personnel and tourists in endemic areas would benefit from an effective HAV vaccine.

Hepatitis A virus is classified as a picornavirus with a single strand positive sense RNA genome coding
20 for a single polyprotein which is subsequently processed into structural and nonstructural proteins. The structural proteins are divided into polypeptides, for example VP1, VP2, VP3 and VP4 which form the capsid polypeptides of the virus. There appears to be
25 only one serotype and significant antigenic variation has not been recognised among different HAV strains.

Replication of the virus in tissue culture is slow and yields are poor, thus making the large scale production for vaccines difficult and expensive.
30 Formalin inactivated (Provost et al, J. Med. Virol., 1986, 19, 23-31. Binn et al., J. Infect. Dis., 1986, 153, 749-756.) and live attenuated virus vaccines (Provost et al., Proc. Soc. Exp. Biol. Med., 1982, 170,

8-14. Karron et al., J. Infect. Dis., 1988, 157, 338-345.) have been produced and shown to be protective both in primates and human volunteers. Problems of large scale production and stability of these vaccines in man need to be overcome and the safety and durability of immunity still have to be established. Moreover, one disadvantage of these vaccines is that 3 doses of vaccine are required to produce an adequate anti-HAV response.

10 A candidate sub-unit vaccine based on the VP1 structural polypeptide has been produced by recombinant DNA techniques in *E. coli* and used to immunize rabbits (Johnston et al., J. Infect. Dis., 1988, 157(6), 1203-1211). The resulting antibody reacted only with 15 denatured VP1 and not with intact HAV, indicating that the conformational epitope had not been expressed by the *E. Coli*.

It has now been found that a valuable vaccine material can be produced by incorporating at least part 20 of the HAV genome in a vaccinia virus, using recombinant DNA technology. The polypeptide expressed by the vaccinia virus is capable of evoking a protective immune response following only a single injection of the material.

25 According to one aspect of the invention there is provided an isolated polypeptide comprising a substantially antigenic part of at least one HAV epitope, free from infectious material.

30 The isolated HAV polypeptide may comprise all or part of VP1, either alone or together with VP3, or all or part of VP3 alone. Alternatively, the polypeptide may comprise all or part of VP1 together with VP4, VP2 and VP3, or at least two HAV structural proteins selected from VP1 to VP4 inclusive.

According to a second aspect of the invention there is provided a DNA molecule comprising a nucleotide sequence substantially coding for all or a portion of at least one HAV structural polypeptide.

5 The nucleotide sequence of the DNA molecule may code for, all or a part of HAV VP1, either alone or together with VP3, or all or part of VP3 alone. Alternatively the nucleotide sequence may code for all or part of VP1 together with VP4, VP2 and VP3, or at
10 least two HAV structural polypeptides selected from VP1 to VP4 inclusive.

The DNA molecule may also comprise a viral promotor operatively linked to the nucleotide sequence.

In yet a further aspect of the invention there is
15 provided a virus genetically engineered to express all or a part of at least one HAV structural polypeptide.

The virus to be genetically engineered may be selected from vaccinia, herpes, papovaviruses such as SV40, papillomaviruses, adenoviruses, retroviruses
20 and baculoviruses, but other viruses not listed here may also be used.

Such genetically engineered recombinant viruses may be incorporated into a vaccine suitable for immunising mammals, in particular man, against hepatitis A
25 infections. Alternatively the vaccine may comprise the proteins expressed by such viruses.

The insertion into a virus, in particular a vaccinia virus, of an HAV cDNA fragment coding for example for the structural proteins VP4, VP2, VP3 and at
30 least some of VP1 (57 amino acids from the amino end) involves preparing the required cDNA using for example the method described by Ticehurst et al in PNAS, 1983,

80, 5885-5889. Alternatively the required cDNA may be derived from one of the recombinant plasmids described by Ticehurst (see above) using restriction endonuclease digestion. In this way, a 1742 base pair cDNA fragment
5 of HAV encoding the structural polypeptides may be readily obtained using Bam HI digestion. Using map positions in Baroudy et al., PNAS, 1985, 82, 2143-2147 a fragment covering positions 611 to 2353 of the HAV genome can be obtained.

10 However, the present invention is not necessarily restricted to the incorporation of such Bam HI fragments of HAV into vaccinia but extends to the incorporation of smaller fragments or different fragments encoding some or all of the VP1, VP2, VP3 and VP4 regions. One
15 combination of particular interest is DNA encoding both the VP1 and VP3 regions, as these regions are believed to contain the immunodominant epitope of the virus, and may also contain the cytotoxic T-cell epitopes. Such DNA fragments can be prepared from larger portions of
20 DNA utilising appropriate restriction enzymes, or alternatively can be synthesised using the polymerase chain reaction with appropriate primers (Bell, Immunology Today, 1989, 10(10), 351-355).

Once prepared, the selected HAV cDNA is inserted
25 into a plasmid vector capable of homologous recombination with a viral genome. Suitable plasmids are described by Mackett et al in J. Virol., 1984, 49(3), 857-864.

The methods for the incorporation of heterologous
30 DNA into vaccinia virus are now well-known and such methods are used in the present invention (see Mackett et al in DNA Cloning ed. Glover (IRL Press, Oxford) Vol. II, 191-211, 1985). Briefly, these involve the introduction of the heterologous DNA encoding the
35 desired HAV epitopes into a plasmid vector of vaccinia

downstream of the vaccinia promoter so that it is flanked by the vaccinia TK sequences and introducing the resultant recombinant vector into cells infected with vaccinia virus. By recombination between the vector
5 containing the HAV sequences and the homologous sequence in the vaccinia genome it is possible to generate a TK recombinant virus which is capable of expressing the heterologous gene. The techniques that have been used for the incorporation of other heterologous genes into
10 vaccinia virus are described for example in Kieny et al, Nature, 1984, 312, 163-166 (1984); Wiktor et al, PNAS 81, 7194-7198 (1984); Moss et al, Nature 311, 67-69(1984); Mackett et al, Science, 227, 433-435 (1985); Elango et al, PNAS, 83, 1906-1910 (1986) and
15 Mackett et al, B.J. Virol. 48, 857-864 (1984); and reference may be made to any of these documents for further guidance.

A particularly convenient method involves transfection of mammalian cells infected with vaccinia
20 virus. The use of the recombinant techniques described results in part of the functional TK gene of the wild type vaccinia virus being replaced by the non-functional TK gene sequence within which is incorporated the DNA encoding the HAV epitope. The recombinant virus is TK-
25 and can therefore be selected with 5-bromodeoxyuridine.

It has been found that when the cDNA fragment of HAV encoding at least one HAV structural polypeptide is expressed under the control of the vaccinia promoter, the expressed polypeptide is not merely the desired HAV
30 capsid polypeptide but is polypeptide that appears to fold in the correct manner to form the conformational epitope.

Most tissue cell lines are capable of supporting the growth of vaccinia virus, for example CV1 cells as
35 well as Vero cells, human lymphoid and diploid cells and

TK- cells (human and mouse) can be used. For large scale production of virus HeLa S3 spinner cells seem to give the best yields.

The recombinant vaccinia virus in accordance with the present invention is capable of expressing HAV polypeptide as a fusion product. There is no termination codon at the end of the 1.7 kb HAV fragment. Transcription continues into the interrupted TK gene until a termination codon is encountered. The expected length of the fusion protein would be about 65000 Daltons, as the interrupted TK gene is in frame with the HAV insert. Translation ends at the TK gene termination codon. This has been confirmed by Westernblot analysis, by immuno-staining of vaccinia infected cell monolayers and by radioimmunoassay. In the latter case positive to negative ratios of 5 were obtained. The HAV proteins expressed by the recombinant vaccinia virus have been found capable of affording protection against subsequent challenge by HAV. The immunity provided by the HAV proteins expressed by the genetically engineered or recombinant vaccinia virus, when injected into a susceptible host, may be both cell mediated, that is T-cell mediated and humoral. Consequently, such HAV proteins are of value in a protective vaccine. The present invention therefore includes a vaccine composition comprising the recombinant vaccinia virus according to the present invention either as inactivated whole or live virus. Alternatively, the vaccine may comprise an isolated HAV as hereinbefore described. Such a vaccine will normally be formulated in a sterile aqueous medium that will be pyrogen-free for parenteral administration, e.g. by the subcutaneous, intradermal, intravenous and intramuscular routes or by scarification.

The purified (virus-free) recombinant antigenic protein expressed in tissue culture by the recombinant

virus can also be used as a vaccine. Similarly, the same gene product for vaccine production (with or without downstream TK sequences) could be inserted into suitable expression vectors for expression in
5 prokaryotic cells or baculoviruses for expression in insect cells.

The recombinant proteins of the present invention are also of use in the production of antibodies, either
10 monoclonal or polyclonal, obtainable by conventional hybridoma or serum raising methods. Such antibodies are useful either in passive immunisation or as diagnostic agents.

In the accompanying drawings :

15 Figure 1 shows the construction of the recombinant vaccinia virus in accordance with Example 1;

Figures 2 and 3 (a), (b) and (c) illustrate the results of testing the recombinant HAV of the invention as a protective vaccine in animal tests as described in
15 Example 2 below.

Figure 4 shows the structural region of HAV genome and chimeras containing coding sequences for the structural polypeptides.

The invention will be further described by way of
20 reference to the following examples :

Example 1

Preparation of Recombinant Vaccinia Virus

An insertion vector, pGS 62 containing a vaccinia virus early promoter (7.5K) (Ticehurst et al., J. Clin. Microbiol., 1987, 25, 1822-1829) was linearised with Bam
25 H1 endonuclease digestion followed by phosphatase

treatment. A Bam HI fragment from plasmid pHAV/J (Karayiannis et al., Viral Hepatitis and Liver Disease ed. Zuckerman (Alan R. Liss New York 1988) 117-120) and covering position 611 to 2353 of the HAV genome, was
5 ligated with the linearised pGS 62 immediately after the 7.5K promotor to form the chimeric plasmid pGS 62/HAV. There were 101 nucleotides for the 5' untranslated region of the HAV genome between the cloning site and the ATG to the HAV open reading frame coding for the
10 polyprotein. The remainder of the genome codes for the structural polypeptides VP4, VP2, VP3 and the first 57 amino acids of the amino terminal end of VP1. E.coli colonies from transformed bacteria carrying the chimeric plasmid were identified by hybridisation to a
15 32P-labelled HAV cDNA probe and the correct orientation of the insert, with respect to the vaccinia 7.5K promotor, was determined by dideoxynucleotide sequencing.

The construction of the chimera is set out in detail in Figure 1 of the accompanying drawings. The
20 resulting chimeric plasmid was transfected into Vero cells in tissue culture using standard procedures. The same cultures were then infected with wild-type vaccinia. Genetic recombination occurs between homologous regions in the plasmid and wild-type
25 vaccinia virus DNA resulting in the interruption of the thymidine kinase (TK) gene. The resultant TK recombinant viruses were selected as described in Mackett et al, Chapter 7, DNA Cloning, Vol. II (D. M. Glover, Ed. IRL Press Oxford, 1985, 191-211). The
30 presence of the foreign gene in the recombinant vaccinia virus was confirmed by dot-blot hybridisation (Karayiannis et al., Viral Hepatitis and Liver Disease, ed. Zuckerman, A. J. (Alan R. Liss, New York) 117-211) and Southern blot analysis (Mackett et al.,
35 see above) using 32P-labelled HAV cDNA probes.

Expression of HAV polypeptides was established by

a solid phase RIA (Karayiannia et al, J. Med. Virol., 1986, 18, 261-276) of cell lysates and by immunostaining of virus infected monolayers with human anti-HAV. Detection of plaques expressing HAV polypeptides was achieved by using rabbit anti-human and swine anti-rabbit antisera in turn. The latter was labelled with biotin and alkaline phosphatase (DAKOPATTS, ABCComplex.AP, Denmark).

Example 2

10 Preparation and Testing of HAV Vaccine

A vaccine was prepared using the recombinant virus obtained as described in Example 1. The vaccine was formulated with recombinant virus suspended in Gibco-BRL's RPMI medium 1640 (a growth medium that does not contain foetal calf serum) so that 0.1 ml of the vaccine contained 10^8 plaque forming units (PFU). The tests were carried out on three tamarins, Saguinus labiatus. Two of the animals were inoculated intradermally with 10^8 PFU in the upper part of their back while the third animal was inoculated with a similar formulation containing 10^7 PFU of wild-type vaccinia virus.

Prior to vaccination, the serum samples from all three animals were negative for anti-HAV antibody. Serum from all three animals was tested again for anti-HAV antibody nine weeks after vaccination, the two animals inoculated with the vaccine of the invention had detectable anti-HAV antibody titres of 1/40 and 1/100.

25 Ten weeks after vaccination, all three animals were challenged intravenously with 0.4 ml live HAV strain HM 175 grown in tissue culture. The disease profile with this inoculum was predetermined in a fourth tamarin, the results being shown in Figure 2 of

the accompanying drawings. Figure 3 of the accompanying drawings shows that tamarin 125, vaccinated with wild-type virus, developed the expected changes similar to those illustrated in Figure 2. The ALT (alanine aminotransferase) elevation lasted from weeks 1 to 3. In contrast, the two animals vaccinated with the vaccine of the invention did not show any ALT elevations. Their ALT levels fluctuated at about their pre-challenge value. The two animals protected with the vaccine of the invention showed no histological changes in their liver biopsies performed three weeks after challenge. Preinnoculation biopsies were normal in all three animals. However, the liver biopsy of the unprotected animal 125, taken during the acute phase of the hepatitis, showed predominantly cytopathic changes.

IgM anti-HAV antibody was detected in the control animal 125 from weeks 2 to 6. No IgM anti-HAV response was detected in the animals protected by the vaccine of the invention, but there was a secondary response following challenge with the live virus. The titres rose to 10^2 and 10^3 for each protected animal respectively within a week of challenge and remained at those levels throughout the period to follow up. In control animal 125, anti-HAV titres did not plateau until week 6.

These results show that the vaccine of the invention not only elicited an immune response to HAV structural polypeptide but subsequently protected the animal when challenged with a live HAV strain capable of inducing hepatitis as demonstrated in the two unprotected animals.

Example 3

Preparation of HAV structural polypeptides

In order to determine the role of the four

structural polypeptides (VP1, VP2, VP3, VP4) of the hepatitis A virus in eliciting an immune response, further chimeras were prepared using the method described in Example 1, for the generation of the
5 relevant recombinant vaccinia viruses.

As shown in Figure 4, chimeras were constructed containing the coding sequence for individual structural polypeptides or combinations of these. The coding sequences were generated using the polymerase chain
10 reaction with the appropriate primers. The 5' end primer contained an initiation codon (ATG) ensuring in frame translation of the relevant polypeptide.

Using chimeras 1), 2) and 5) recombinant vaccinia viruses were generated and tested for immunogenicity by
15 injection into rabbits and by radioimmunoassay using polyclonal antibodies.

Prior to inoculation with recombinant vaccinia virus, serum samples from all rabbits are negative for anti-HAV antibody. Serum from all rabbits is tested
20 again for anti-HAV antibody and all show detectable anti-HAV antibody to varying degrees.

Example 4

Detection of cell mediated immunity

Cell lines, either fibroblast or lymphoblast cells
25 from tamarins are established using known techniques and then transformed. Following establishment, the cell lines are infected with various of the recombinant viruses shown in Figure 4.

Lymphocytes from tamarins infected with virulent
30 HAV virus are obtained and added to cultures of the cell lines described above. Release of radioactive chromium from the target cells is indicative of cytotoxic T-cell

mediated mechanisms. In order to detect the presence of cell mediated immunity, cell killing is looked for, as evidenced by the release of labelled chromium.

Killing of the lymphocytes from infected tamarins is detected to varying degrees by cell lines infected with recombinants 1, 2, 3, 4 and 5.

CLAIMS

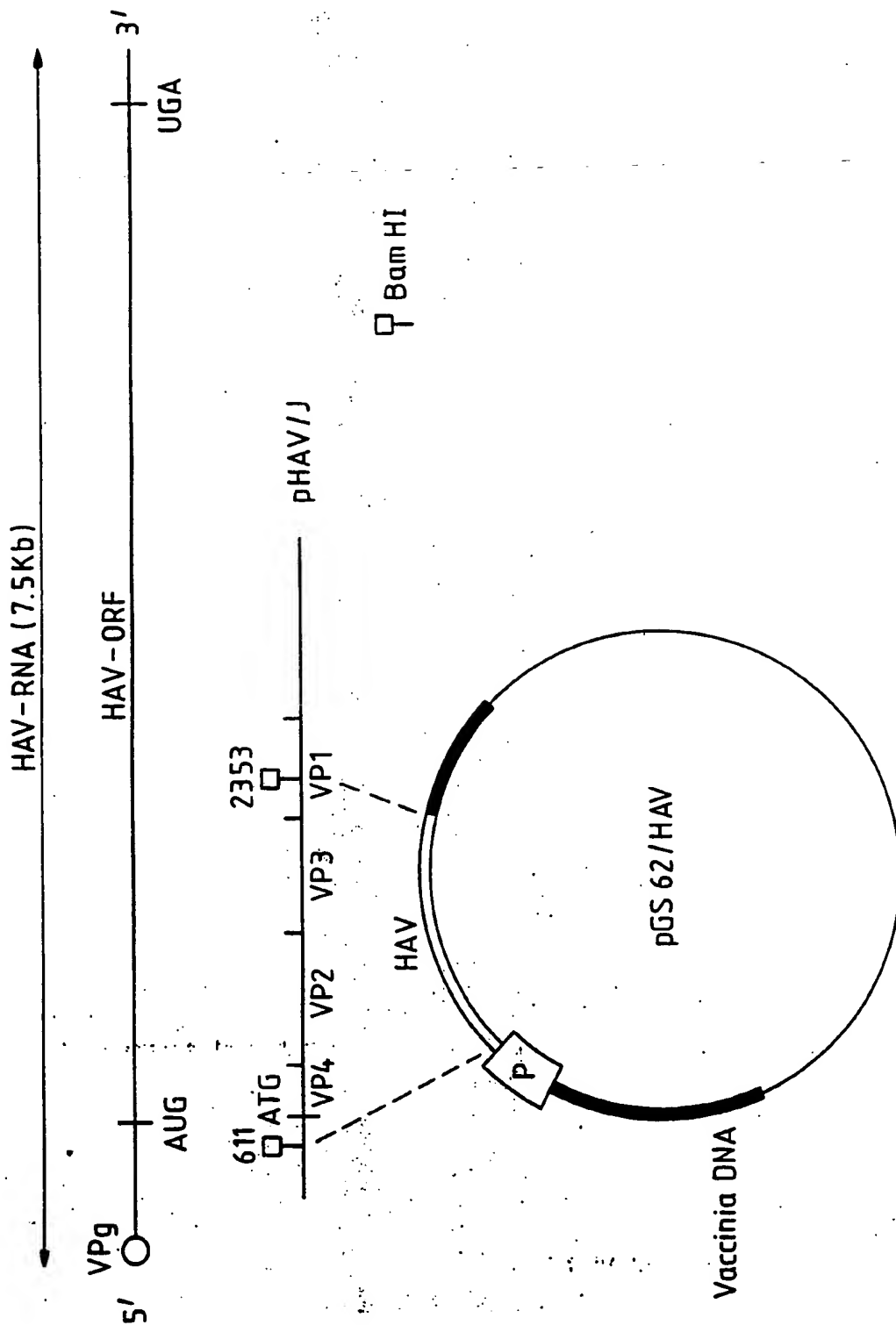
1. An isolated polypeptide comprising a substantially antigenic part of at least one hepatitis A virus (HAV) epitope, free from infectious material.
2. A polypeptide as claimed in claim 1 comprising at least part of HAV VP1.
3. A polypeptide as claimed in claim 1 comprising at least part of HAV VP3.
4. A polypeptide as claimed in any one of claims 1 to 3 comprising HAV VP3 and at least part of HAV VP1.
5. A polypeptide as claimed in any one of claims 1 to 4 comprising HAV VP4, VP2, VP3 and at least part of VP1.
6. A polypeptide as claimed in any of the the preceding claims comprising all or a part of at least two HAV structural polypeptides selected from VP1, VP2, VP3 and VP4.
7. A polypeptide as claimed in any one of the preceding claims wherein the HAV epitope is conjugated to at least a part of thymidine kinase of viral origin.
8. A polypeptide as claimed in claim 7 wherein the thymidine kinase is of vaccinia virus origin.
9. A DNA molecule comprising a nucleotide sequence substantially coding for all or a portion of at least one HAV structural polypeptides.

10. A DNA molecule as claimed in claim 9 wherein the nucleotide sequence substantially codes for at least part of HAV VP1.
11. A DNA molecule as claimed in claim 9 wherein the nucleotide sequence substantially codes for at least part of HAV VP3.
12. A DNA molecule as claimed in any one of claims 9 to 11 wherein the nucleotide sequence substantially codes for at least part of HAV VP1 together with HAV VP3.
13. A DNA molecule as claimed in claim 9 wherein the nucleotide sequence substantially codes for HAV VP4, VP2, VP3 and at least part of VP1.
14. A DNA molecule as claimed in claim 9 wherein the nucleotide sequence substantially codes for all or a part of at least two HAV structural polypeptide selected from VP1, VP2, VP3 and VP4.
15. A DNA molecule as claimed in any one of claims 9 to 14 comprising a viral promotor operatively linked to the nucleotide sequence.
16. A DNA molecule as claimed in claim 15 wherein the viral promotor is derived from vaccinia virus.
17. A virus genetically engineered to express all or a part of at least one HAV structural polypeptide.
18. A virus as claimed in claim 17 capable of expressing a polypeptide according to any one of claims 2 to 8.
19. A virus genetically engineered to contain a DNA molecule as claimed in any one of claims 9 to 16.

20. A recombinant virus capable of expressing all or a part of at least one HAV structural polypeptide.
21. A recombinant virus as claimed in claim 20 capable of expressing a polypeptide according to any one of claims 2 to 8.
22. A recombinant virus as claimed in claim 21 capable of expressing the polypeptide in a host cell under the control of a viral promoter.
23. A recombinant virus as claimed in any one of claims 20 to 22 comprising vaccinia virus.
24. A recombinant virus comprising a DNA molecule as claimed in any one of claims 9 to 16.
25. A recombinant virus as claimed in claim 24 comprising vaccinia virus.
26. A vaccine for immunising against HAV comprising a polypeptide as claimed in any one of claims 1 to 8 in association with a pharmaceutically acceptable carrier.
27. A vaccine for immunising against HAV comprising a virus as claimed in any one of claims 17 to 25 in association with a pharmaceutically acceptable carrier.

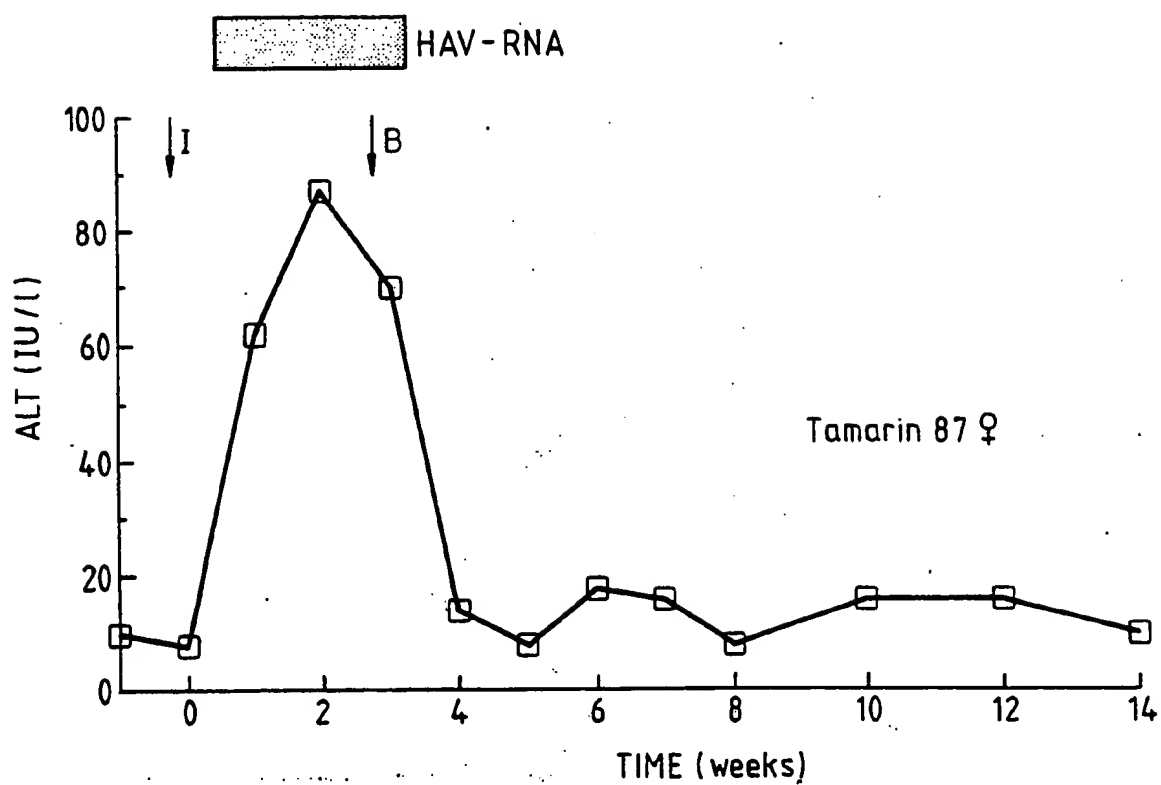
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Fig. 1.



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Fig.2.



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Fig. 3A.

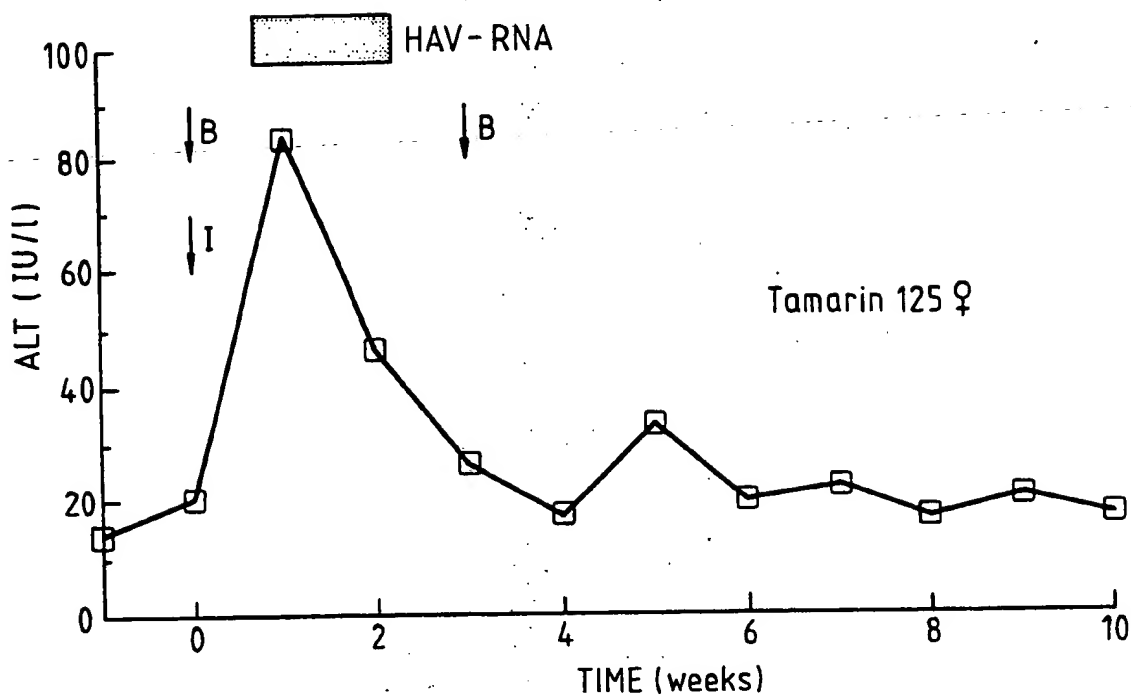
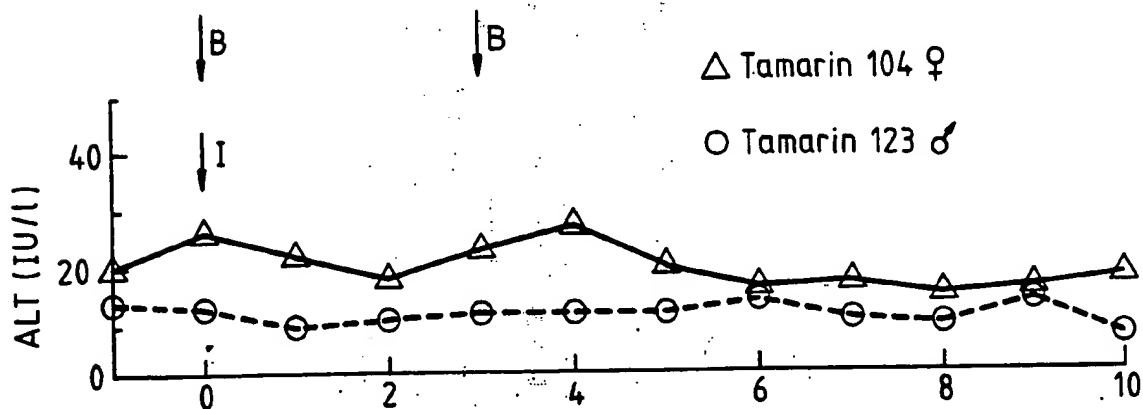


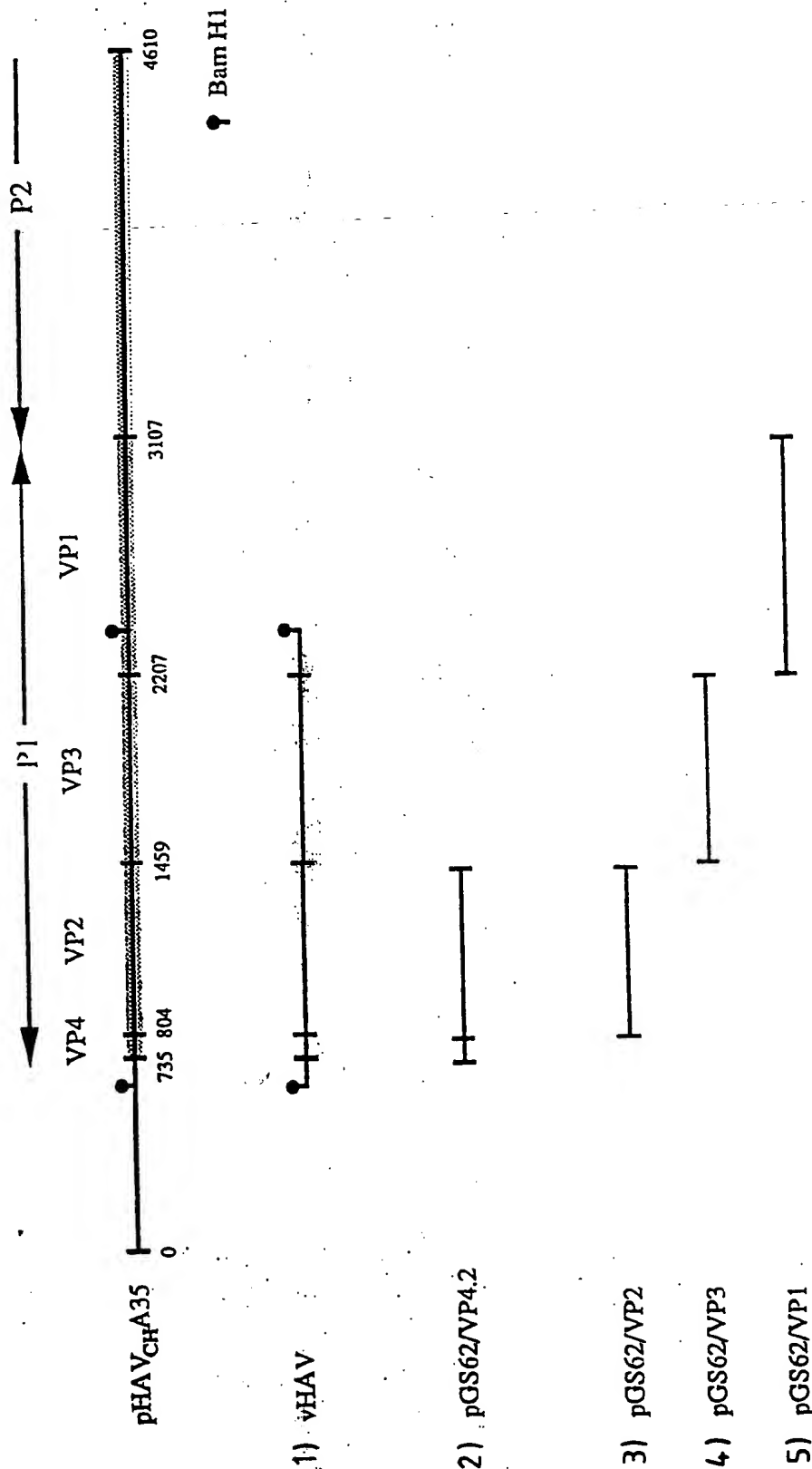
Fig. 3B.



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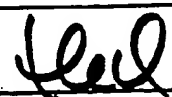
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Fig. 4.



INTERNATIONAL SEARCH REPORT

International Application No. PCT/GB-91/00163

I. CLASSIFICATION OF SUBJECT MATTER (If several classification symbols apply, indicate all) ⁴ According to International Patent Classification (IPC) or to both National Classification and IPC IPC ⁵ : C 07 K 13/00, C 12 N 15/51, 15/86, 7/01, A 61 K 39/29, 39/285		
II. FIELDS SEARCHED Minimum Documentation Searched ⁷		
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Documentation Searched other than Minimum Documentation to the extent that such Documents are included in the Fields Searched ⁸		
III. DOCUMENTS CONSIDERED TO BE RELEVANT⁹		
Category ¹⁰	Citation of Document, ¹¹ with indication, where appropriate, of the relevant passages ¹²	Relevant to Claim No. ¹³
X	EP, A, 0276330 (INSTITUT BIOORGANICHESKOI KHIMII) 3 August 1988 see page 3, line 34 - page 4, line 27; page 5, line 35 - page 6, line 13; examples 8-10; claim	1-27
X	Biological Abstracts, vol. 90, F. Gao et al.: "Expression of hepatitis A virus proteins by recombinant vaccinia virus", see abstract 28501 & Chin. J. Virol. 5 (4), 1989, 303-311	1-27
X	EP, A, 0138704 (MERCK & CO. INC.) 24 April 1985 see claims	1,2,26
<p>¹⁰ Special categories of cited documents:</p> <p>"A" document defining the general state of the art which is not considered to be of particular relevance</p> <p>"E" earlier document but published on or after the international filing date</p> <p>"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</p> <p>"O" document referring to an oral disclosure, use, exhibition or other means</p> <p>"P" document published prior to the international filing date but later than the priority date claimed</p> <p>"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</p> <p>"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step</p> <p>"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents; such combination being obvious to a person skilled in the art.</p> <p>"Z" document member of the same patent family</p>		
IV. CERTIFICATION		
Date of the Actual Completion of the International Search 13th May 1991		Date of Mailing of this International Search Report 11. 07. 91
International Searching Authority EUROPEAN PATENT OFFICE		Signature of Authorized Officer F.W. HECK 

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)		
Category *	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No.
X	EP, A, 0154587 (MERCK & CO. INC.) 11 September 1985 see page 22 - page 25, line 15; claims --	1-3,6,9-11, 14,26
X	Virus Research, vol. 10, 1988, Elsevier Science Publishers B.V., S.A. Harmon et al.: "Expression of hepatitis A virus capsid sequences in insect cells", pages 273-280 see the whole article --	1-4,6,9-12, 14,15,17- 22,24,26,27
A	Proceedings of the National Academy of Sciences of the USA, vol. 82, no. 9, May 1985, (Washington, US), R. Najarian et al.: "Primary structure and gene organization of human hepatitis A virus", pages 2627- 2631 --	
A	J. Gen. Virol., vol. 67, no. 10, 1986, SGM, (GB) M. Mackett et al.: "Vaccinia virus expression vectors", pages 2067- 2082 -----	

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO.

GB 9100163

SA 44447

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report. The members are as contained in the European Patent Office EDP file on 18/06/91. The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP-A- 0276330	03-08-88	SU-A- 1469856	30-09-90
		JP-T- 1500485	23-02-89
		WO-A- 8800973	11-02-88
EP-A- 0138704	24-04-85	US-A- 4614793	30-09-86
		JP-A- 60104020	08-06-85
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